CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
CYTOKININ and 6-Benzyladenine [Promalin]

Chemical Codes # 2082 and 2000, Tolerance # 01042 and 376
SB 950 # 251 and 828
Original date: March 26, 2002

I. DATA GAP STATUS

Combined, rat :	Data gap, study not submitted
Chronic toxicity, rat:	Data gap, study not submitted
Subchronic, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	Data gap, study not submitted
Subchronic, dog:	Data gap, inadequate study
Oncogenicity, rat:	Data gap, study not submitted
Oncogenicity, mouse:	Data gap, study not submitted
Reproduction, rat:	Data gap, inadequate study, no adverse effect indicated
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	Data gap, study not submitted
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	Data gap, inadequate study, no adverse effect indicated
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 116119 in 1042 - 020 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: T020326
Original: J. Kishiyama and Gee, 3/26/02

Cytokinins, considered biopesticides, are plant growth regulators. When derived from seaweed**,** the mixture of four similar substances is exempt from tolerance. 6-Benzyladenine is similar to cytokinin but synthetic and is also exempt from tolerance when used as a fruit-thinning agent at a rate not to exceed 30 g/acre (6 FR 34869, July 5, 1995). The studies on file were conducted with 6-benzyladenine [Promalin].

See: “Reregistration Eligibility Decision (RED): Cytokinin”, US EPA, December 1995, and “Reregistration Eligibility Decision (RED): N6-Benzyladenine”, US EPA, June 1994.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

No study submitted

CHRONIC TOXICITY, RAT

Subchronic:

** 1042 - 020 116119 Salamon, C. M. “13 Week Dietary Toxicity with 6-Benzyladenine in Rats.” (Hazleton Wisconsin, Inc., Laboratory Project ID HWI 6161-117, May 8, 1992.) Technical 6-benzyladenine (99%) was admixed with the feed at concentrations of 0, 500, 1500 or 5000 ppm and fed for 13 weeks to 20, 10, 10, and 20 Crl:CD®BR rats/sex/group, respectively. These doses were equivalent in males to 0, 34.12, 101.5 and 294.78 mg/kg and in females, 0, 41.20, 119.88 and 322.26 mg/kg. Ten of the control and high dose animals were continued on control diet until week 18 for recovery. High-dose animals (M and F) and mid-dose females had significantly lower body weight and food consumption. During the recovery period, the high dose animals gained considerably more weight than the controls and had comparable food consumption. Animals at the high dose appeared thin, hunched and had decreased feces. Lower glucose and elevated hemoglobin, hematocrit, urea nitrogen, inorganic phosphorus and potassium associated with renal injury (postrenal azotemia) for the high dose group were considered treatment-related effects**.** Body weight NOEL = 500 ppm/day. Histological NOEL = 1500 ppm/day, based on kidney findings. ACCEPTABLE with no adverse effects. (Kishiyama and Gee, 3/21/02).

376 - 001 32320 Newell, G. W. “Study of acute and subacute toxicity of Shell compound SD4901.” (Stanford Research Institute, January 15, 1959) The report contains summaries of a range-finding study and a 90-day study in rats. Range-finding: 4 Long-Evans rats/sex/group were fed SD4901 in the diet for 3 weeks at 295, 925, 2950 or 9250 ppm. Diets of 2950 and 9250 ppm resulted in lower food consumption and decreased body weight. From food efficiency at 2950 ppm in males, the conclusion was that food at 9250 ppm was unpalatable, rather than due to direct toxicity of 6-benzyladenine. Subacute: Ten rats/sex/group were fed diets containing 0, 80, 200, 500, 1270 or 3200 ppm for 90 days. At weeks 6 and 13, blood samples were drawn for limited hematological parameters. Body weights were measured weekly. Growth at 3200 ppm was lower than other groups, primarily early in the study. After week 6 or 7, growth rates were comparable. Palatability was a possible cause. Hematological values were within normal range. Gross pathology showed respiratory infection in all groups but no treatment-related pathology. Organ weights (lung, liver, kidney, heart, spleen) were comparable. Examination of tissues (limited list) from 3/sex from controls, 500, 1270 and 3200 ppm did not reveal treatment-related changes. No adverse effects from a limited study. UNACCEPTABLE due to limited parameters. Not upgradeable. No worksheet. (Gee, 3/22/02)

Note: Study in 32320 confirms the finding in study in 116119 that palatability at higher doses was a probable cause of some effects on body weight and food consumption, rather than specific toxic effects on organs. (Gee, 3/22/02)

CHRONIC TOXICITY, DOG

No study submitted

Subchronic:

376 - 001 32315 Shellenberger, T. E., C. Mitoma and G. W. Newell "Study of subacute toxicity and metabolism of Shell compound SD 4901." (Stanford Research Institute, September 15, 1961) Part I reported on a 90-day study with beagle dogs, 2 males and 3 females per group, fed diets containing 0, 125, 400 or 1200 ppm SD 4901 (6-benzyladenine, >99%), equivalent to mean intakes of 0, 3, 9 and 29 mg/kg/day for males and 0, 4, 11 and 28 mg/kg/day for females. Blood samples were taken pre-test and weeks 4, 8 and 12 for limited hematological parameters. No difference in weight gain was noted in any group. There were no affects on hematocrit, hemoglobin, RBC counts or WBC counts. Organ weights were comparable. Although no data were presented, the report states that microscopic examination of control and 1200 ppm dogs did not show evidence of treatment-related findings. Part II of the report summarized results of metabolism of labeled compound in rats and dogs. With dogs, the compound (15 mg with label as benzyl-1-C^{14}-adenine) was fed in a small ball of meat. Urine was collected for 24 and 48 hours. Essentially all of the radioactivity was excreted in urine in 24 hours. By co-chromatography, three metabolites were identified as hippuric acid, benzoic acid and benzyladenine (the same as in the rat). With animals given benzyladenine-8-C^{14}, the profile was somewhat different and components were not identified except for parent compound. Excretion of the adenine label was slower. Profiles of rat and dog were, again, similar. No adverse effects. Apparent NOEL > 1200 ppm. UNACCEPTABLE and not upgradeable due to deficiencies in the protocol, no individual data, others. No worksheet. (Gee, 3/22/02)

ONCOGENICITY, RAT

No study submitted

ONCOGENICITY, MOUSE

No study submitted

REPRODUCTION, RAT

376 - 001 32316 Plotnikoff, N., C. Mitoma, and G. W. Newell. "Metabolism and Reproduction Studies of Shell Compound SD4901." (Stanford Research Institute, October 27,1959.) Part I of the report contained results of studies on metabolism. In brief, approximately 96% of radioactive 6-benzyladenine was accounted for in urine and feces in 4 days. The highest retention was in the liver. The major urinary metabolite was hippuric acid. Part II summarized a study on parturition of rats. Benzyladenine was admixed with feed at concentrations of 0, 500, 1000 or 2000 ppm and fed to 9 female rats/group for 2 weeks. These females had previously mated and produced a normal litter. Females were mated with males for 1 - 3 days and repeated until females were pregnant as noted by increased body weight. Pup weight, number, viability at 0, 4 and 21 days were recorded. No maternal mortality or other effects and no significant effects on pregnancy, birth, pup weight or survival. Insufficient information to determine a NOEL but possibly > 2000 ppm. No work sheet. UNACCEPTABLE and not upgradeable with no adverse effects. (Kishiyama and Gee, 3/22/02)

TERATOLOGY, RAT

** 1042 - 019 114204 Hui, J. Y. "Evaluation of the Effects of Orally Administered 6-Benzyladenine (Abbott-39313) on the Embryonic and Fetal Development of the Rat (Segment II TFR)." (Abbott Laboratories, Laboratory Project ID TA90-007, August 9, 1990.) 6-Benzyl adenine (99.2%) was administered via gavage at doses of 0 (0.2% hydroxypropylmethyl cellulose), 15, 50, or 175 mg/kg to 25 pregnant rats/group during days 6 through 15 of gestation. Food consumption, body weight and body weight gain were reduced for the high dose maternal group. Maternal NOEL = 50 mg/kg/day. Fetal weight was reduced 10% for the high dose group. No malformations or other fetal effects were noted. Developmental NOEL = 50 mg/kg/day. ACCEPTABLE with no adverse effects. (Kishiyama and Gee, 3/20/02).

1042 - 006 903093 Teratology. Accel Technical was administered at concentrations of 0, 32, 100 or 320 mg/kg during gestation days 6 through 15 to 19-20 female rats/group. UNACCEPTABLE (insufficient information for assessment). (D.S. and J. Wong; 6/27/85).

TERATOLOGY, RABBIT

No study submitted

GENE MUTATION

** 1042 - 019 114202 Jagannath, D. R. " Mutagenicity Test on 6-Benzladenine in the Ames *Salmonella*/Microsome Reverse Mutation Assay." (Hazleton Laboratories of America, Inc., Laboratory Project ID 9975-0-401, November 3, 1987.) 6-Benzyladenine 16262 (purity 99.6%) was evaluated for mutagenicity at concentrations of 0, 5, 10, 50, 100, 500, 1000, 2000, and 4999 :g/plate using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 with and without rat liver metabolic activation (S-9 Mix). There was a single trial with triplicate plates per concentration. 6-Benzyladenine treatment with and/or without metabolic activation (S9 Mix) did not significantly increase the number of revertants in *Salmonella typhimurium* tester strains. Positive controls were functional. No adverse effect. ACCEPTABLE. (Kishiyama and Gee, 3/18/02)

CHROMOSOME EFFECTS

** 1042 - 019 114200, 114201 Ivett, J. L. AMutagenicity Test on 6-Benzyladenine 16262 in the *In Vivo* Mouse Micronucleus Assay. A (Hazleton Laboratories of America, Inc., Laboratory Project ID: 9975-0-455, November 4, 1987.) 6-Benzyladenine (99.6%) was administered once by oral gavare at doses of 0 (0.5% CMC), 140, 467 or 1400 mg/kg to 5 ICR mice/sex/group for each scheduled sacrifice time (24, 48, and 72 hours after dosing). Positive control was Triethylenemelamine and was functional. There was mortality at the high dose (80% of the LD50), especially for males. 6-Benzyladenine treatment did not significantly increase the percentage of polychromatic erythrocytes. No adverse effects. ACCEPTABLE. (Kishiyama and Gee, 3/18/02).

DNA DAMAGE

1042 - 019 114203 Cifone, M. A. "Mutagenicity Test on 6-Benzyladenine in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay." (Hazleton Laboratories of America, Inc., Laboratory Project ID 9975-0-447, January 13, 1988.) 6-Benzyladenine 16262 (purity 99.6%) was evaluated at concentrations of 0 (DMSO), 1, 2.5, 5, 10, 25, 50, 100, 250 and 500 :g/ml using rat primary hepatocytes. Five replicate cultures were exposed for 18 - 19 hours. Concentrations of 100 ug/ml and higher were too toxic for evaluation. UDS was evaluated by autoradiography with 50 cells per slide from triplicate cultures scored for net nuclear grains. The other two cultures were used for cytotoxicity by trypan blue dye exclusion. Cells were examined microscopically. Test article 6-Benzyladenine did not induce significant changes in the nuclear labeling of rat primary hepatocytes. UNACCEPTABLE. Upgradeable (more details of data, including cytoplasmic counts and nuclear grain counts per slide for better evaluation of the results). (Kishiyama and Gee, 3/19/02).

NEUROTOXICITY

Not required at this time.

OTHER

NOTE: The following are "one-liners" of 6-Benzyladenine (Chem code 2000; DPN# 376; SB950# 828) acute studies.

ACUTE STUDIES

Acute Oral

376 - 007 128845 Cozzi, E. M. "Acute Oral Toxicity Study in Rats with 6-Benzyladenine, Drug Safety Study No. 76-278." (Abbott Laboratories, 5/18/90.) 6-Benzyladenine (99% purity) was administered via single gavage at doses of 0.94, 1.2, 1.5, 1.9, 2.4 or 3.0 ?/kg to 10 Long-Evans rats/sex/group. Decreased activity and increased activity, ataxia, dyspnea, tremors and cyanosis were reported on Day 1 of the 14 day study period. Mortality for males was 1/10, 5/10, 8/10, 8/10, 9/10, and 10/10; for females 0/10, 6/10, 8/10, 8/10, 8/10, and 9/10 at the above respective doses. Unacceptable (insufficient information). A two page summary of a study. No worksheet. (Kishiyama and Gee, 3/22/02).

Acute Dermal Toxicity

376 - 007 128848 Cozzi, E. M. "Acute Dermal Toxicity Study in Rabbits with 6-Benzyladenine, Drug Safety Study No. 76-279." (Abbott Laboratories, 5/18/90) 6-Benzyl adenine (99% purity) was administered to 10/sex New Zealand albino rabbits at a dose of 5 g/kg for 24 hours under occlusive dressing. Decreased activity, ataxia, dyspnea, and paralysis (males only) were seen only on day 1 and similarly for day 2 except the absence of paralysis. Mortality was 2/10 for both males and females (time of death was not reported) Unacceptable (insufficient information). A summary only. No worksheet. (Kishiyama and Gee, 3/22/02).

Acute Inhalation LC50:

** 376 - 007 128851 Hoffman, G. M. “An Acute Inhalation Toxicity Study of 6-Benzyladenine in the Rat”. (Bio/dynamics Inc., Laboratory Project ID 89-8248, August 20, 1990.) 6-Benzyl adenine (99.0% pure) was administered as a dust by whole body inhalation at analytical concentrations of 1.2, 4.7, 6.2 , 4.6 or 7.4 mg/l for a single 4 hour exposure to 5 Sprague-Dawley CD® rats/sex/group. Data from the 4.7 and 7.4 mg/l were excluded due to the high variability in samples. Animals were observed for 14 days. Labored breathing was the most notable sign for all groups except for group V (1.2 mg/l). The mass median aerodynamic diameter ranged from 2.6 to 6.2 microns and the geometric standard deviation ranged from 2.0 to 5.7 microns. At least 68% of particles were 10 microns or less. LC_{50} for males = 5.1 (3.1 to 8.5) and females = 5.3 (2.9 to 9.6) mg/l. Combined sexes LC50 was 5.2 mg/l. Toxicity Category III. ACCEPTABLE. (Kishiyama and Gee, 3/22/02).

Eye Irritation:

376 - 007 128849 Cozzi, E. M. “Primary eye irritation study in rabbits with 6-benzyladenine, Drug Safety Study No. 76-305.” (Abbott Laboratories, 5/18/90) 6-Benzyladenine (99%) was introduced into the conjunctival sac of 8 New Zealand albino rabbits at 50 mg per animal as a fine powder. The eyes of 3 rabbits were unwashed and 5 were washed 5 minutes after treatment. Eyes were examined at 5 minutes and 24, 48 and 72 hours. An average score of “1" was noted for conjunctivitis at 5 minutes and 24 hours, then subsided by 48 hours. Scores for corneal opacity and iritis were “0" at all observation times. UNACCEPTABLE (use of 50 mg, only 3 animals.) No worksheet - not the complete study. (Gee, 3/22/02)

Dermal Irritation

** 376 - 007 128852 Shults, S. K. “Primary Dermal Irritation Study in Albino Rabbits with 6-Benzyladenine.” (Ricerca, Inc., Laboratory Project ID 91-0095, Document Number 3882-91-0095-TX-001, May 22, 1991.) A dose of 0.5 g of 6-benzyladenine (99.0% pure), slightly moistened with 0.5 ml water, was applied once to the clipped skin for 4 hours to 3 New Zealand White rabbits/sex. The site was covered. Skin was examined and scored for irritation at 30 to 60 minutes, 24, 48 and 72 hours and days 4, 5 and 6. Very slight to well defined erythema was present in all 6 rabbits at 30 to 60 minutes and in 3 rabbits at 24 hours. Very slight erythema was seen in 3 rabbits at 48 and 72 hours and persisted in 2 rabbits through day 5. All were clear after day 5. No edema was reported. The test article was considered a mild to slight irritant over 72 hours with a primary irritation index of 0.7. Category IV. ACCEPTABLE. (Kishiyama and Gee, 3/25/02).

Sensitization

** 376 - 007 128853 Kreuzmann, J. J. “Delayed Contact Hypersensitivity Study in Guinea Pigs of: 6-Benzyladenine”. (Hill Top Biolabs, Inc., Laboratory Project ID 90-4028-21, Document 12, August 14, 1990.) 6-Benzyladenine, at a concentration of 25% (w/v in acetone), was applied dermally (6 hrs) to 10 guinea pigs/sex (induction 3x + challenge 1x) and 5 Guinea pigs/sex (naive control [1x challenge]). No evidence of sensitization. No significant effect on body weight. ACCEPTABLE. (Kishiyama and Gee, 3/25/02).